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13. ABSTRACT (Maximum 200 Words)

A goal of this proposal is to achieve a high intratumoral concentration of 5-fluorouracil (5-FU) *via* molecular chemotherapy employing genetically modified adenoviral (Ad) vectors encoding the genes for somatostatin receptor subtype 2 (SSTr2) and cytosine deaminase (CD) which converts the prodrug 5-fluorocytosine (5-FC) to 5-FU under control of the cyclooxygenase-2 (Cox-2) tumor-specific promoter. The purpose of Specific Aim 1 was to develop, validate, and evaluate genetically modified Ad vectors that will increase expression levels of both SSTr2 and CD. We proposed to initially evaluate two novel two-gene Ad vectors: (1) a native fiber Ad (AdCMVCDCMVSSTr2) and (2) Ad under control of the Cox-2 promoter expressing CD and SSTr2. We have produced several vectors including AdCox-2LCDCox-2LSSTr2, AdCox-2LSSTr2Cox-2LCD, AdRGDCox-2LCDCox-2LSSTr2, and AdRGDCox-2LCDCox-2LSSTr2. The vectors we developed were tested for SSTr2 and CD expression employing membrane receptor binding *in vitro* with ^{125}I-somatostatin and ^{99m}Tc-P2045 that binds to SSTr2, conversion of 5-FC to 5-FU, and cytotoxicity against Ad infected cells in the presence of 5-FC. The vectors were evaluated *in vivo* for SSTr2 expression and CD expression. Efforts are continuing to produce RGD modified vectors expressing CD and SSTr2 under control of the Cox-2L promoter to be used for therapy in local and metastatic models.

14. SUBJECT TERMS

Prostate cancer, gene therapy, molecular chemotherapy, transcriptional targeting, cytosine deaminase, somatostatin receptor, brachytherapy

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INTRODUCTION

The objective of this project is to develop novel two gene adenovirus (Ad) vectors expressing human somatostatin receptor subtype 2 (SSTr2) and cytosine deaminase (CD) under control of the cyclooxygenase-2 (Cox-2) promoter for radiolabeled peptide therapy/molecular chemotherapy of prostate cancer. The tumor specific promoter Cox-2 was selected based on the fact that it is active in prostate cancer, and the liver is Cox-2 negative and is the predominant site of Ad vector localization after systemic administration. Thus, the Ad may infect normal liver cells but no transgene expression would occur.

Two forms of the Cox-2 promoter were compared to the cytelomegalovirus (CMV) promoter in the previous project period; a "long" form (Cox-2L) and a truncated "medium" form (Cox-2M). It was shown that the Cox-2L promoter resulted in greater CD activity than the Cox-2M promoter so in the studies conducted during the current project period we focused on the Cox-2L promoter. A goal was to determine if transductional targeting with the RGD peptide in the HI-loop of the knob of the Ad vector would result in greater SSTr2 and CD expression. Moreover, the new vectors were evaluated *in vivo* for SSTr2 and CD expression in prostate cancer xenografts.

BODY

Cellular Cox-2 mRNA status and promoter activity was determined in LNCap, DU145, and PC3 human prostate cancer cells using Ad vectors expressing luciferase. DU145 and PC3 cells expressed Cox-2 mRNA and their Cox-2L promoter activity was 70% and 100% of CMV promoter activity (**Figure 1**).

Seven vectors expressing CD and/or SSTr2 were developed and evaluated using the prostate cancer cell lines DU145 and PC3: AdCox-2LCDCox-2LSSTr2, AdCox-2LSSTr2Cox-2LCD, AdRGDCox-2LCDCox-2LSSTr2, AdCMVCDCMVSSTr2, AdCMVCD, AdRGDCMVCD, and AdRGDCMVCDCMVSSTr2. Somatostatin binding and internalization assays were performed by incubating infected cells with ^{99m}Tc-P2045, a peptide specific for SSTr2, and gamma camera imaging of cells in plates 5 min after addition of peptide and 20 min later after removing the excess peptide and stripping the peptide bound to SSTr2 on the cell surface.[1,2] As shown in **Figure 2**, DU145 prostate cancer cells infected with AdCMVSSTr2, AdCox-2LCDCox-2LSSTr2, or AdCox-2LSSTr2Cox-2LCD showed binding and internalization of ^{99m}Tc-P2045, demonstrating that the vectors all induced SSTr2 expression, although the Cox-2L vectors produced less SSTr2 expression than the CMV vector. In addition, AdCox-2LSSTr2Cox-2LCD produced more SSTr2 expression than AdCox-2LCDCox-2LSSTr2.

Athymic nude mice bearing DU145 prostate cancer xenografts were injected intratumorally with AdCMVSSTr2 or AdCox-2LSSTr2 followed 2 days later by *i.v.* injection of ^{99m}Tc-P2045. As shown in **Figures 3** and **4**, there was less accumulation of ^{99m}Tc-P2045 in tumors injected with AdCox-2LSSTr2 compared to AdCMVSSTr2. Scatchard analysis demonstrated higher SSTr2 expression in DU145 and PC3 cells infected with the RGD modified AdRGDCMVCDCMVSSTr2 than following infection with AdCMVCDCMVSSTr2 or AdCMVSSTr2 (**Figure 5** and **Table 1**). These results indicate that the Cox-2 vectors produced less SSTr2 expression than the CMV vector both *in vitro* and *in vivo*, and indicate the importance of producing RGD modified Cox-2 vectors.

Table 1. Binding of ^{125}I-somatostatin to human prostate cancer cell lines 48 h after infection with 100 MOI of each virus.

	DU145		PC3	
	B_{max}	fmol/μg	B_{max}	fmol/μg
AdCMVSSTr2	364913	4.4	125852	1.5
AdCMVCDCMVSSTr2	238182	2.7	93592	1.1
AdRGDCMVCDCMVSSTr2	501701	5.6	276868	3.1

CD conversion assays were performed with lysates from infected cells with the addition of ^{3}H-5-fluorocytosine (5-FC) at various time points, and separation on thin layer chromatography plates in butanol and water.[3] Spots containing 5-FC and 5-fluorouracil (5-FU) were cut out, put into scintillation fluid, and counted in a liquid scintillation counter. Cytotoxicity assays were performed using the MTS assay (Promega) as described in the manufacturer's protocol. CD expression in tumor xenografts was detected by immunohistochemistry. CD conversion assays were also performed with lysates from prostate cancer xenografts. Tumors were homogenized and lysed, and CD conversion assays performed as described for cellular lysates.

The vectors expressing CD and SSTr2 were next tested to determine their conversion of 5-FC to 5-FU following infection of DU145 and PC3 human prostate cancer cell lines. The results are presented in **Table 2**.[3] They indicate that AdCox-2LCD, AdCox-2LCDCox-2LSSTr2, and AdCox-2LSSTr2Cox-2LCD produced a lower level of conversion of 5-FC to 5-FU in DU145 and PC3 cells than AdCMVCD and AdCMVCDCMVSSTr2. Furthermore, AdRGDCMVCD produced a level of conversion that was significantly greater than AdCMVCD. The highest level of CD expression in DU145 tumor xenografts occurred following infection with AdCMVCD as compared to AdCox-2LCDCox-2LSSTr2 or AdCox-2LSSTr2Cox-2LCD (**Figure 6**). In addition, the Cox-2L CD and SSTr2 vectors produced a lower level of 5-FC to 5-FU conversion as compared to AdCMVCD (**Figure 7**). These results support earlier ones that the CMV promoter is stronger than the Cox-2 promoter, although less specific. Moreover, the RGD modified CMV viruses produced the highest level of conversion, whereas the RGD modified Cox-2L viruses (AdRGDCox-2LCD and AdRGDCox-2LCDCox-2LSSTr2) did not produce CD, suggesting a problem in their design.

Table 2. Conversion of 5-FC to 5-FU in pmol/min/mg measured over a 1 h period at 48 h after infection of DU145 and PC3 human prostate cancer cell lines with 100 MOI of each virus.

Vector	DU145 (pmol/min/mg)	PC3 (pmol/min/mg)
Uninfected	-0.01	0.00
AdCMVCD	25.6	8.1
AdCMVSSTr2	0.04	-0.02
AdCMVCDCMVSSTr2	7.5	2.5
AdCox-2LCD	2.1	1.0
AdCox-2LCDCox-2LSSTr2	3.0	1.2
AdCox-2LSSTr2Cox-2LCD	2.5	0.9
AdRGDCMVCD	150.4	194.0
AdRGDCMVCDCMVSSTr2	38.5	35.6
AdRGDCox-2LCD	1.1	0.5
AdRGDCox-2LCDCox-2LSSTr2	0.1	0.04

In the next set of studies, we evaluated the cytotoxicity of the various vectors after infection of DU145 cells with 10 MOI (plaque forming units/cell) of virus. The 5-FC IC_{50} values are shown in **Table 3**. The results indicate that AdCox-2LCDCox-2LSSTr2 produced equivalent cytotoxicity compared to AdCMVCD, whereas AdCMVCDCMVSSTr2, AdRGDCMVCD, and AdRGDCMVCDCMVSSTr2 produced the greatest degree of cytotoxicity. The level of cytotoxicity was lowest in the AdRGDCox-2LCD and AdRGDCox-2LCDCox-2LSSTR2 infected cells. These results are consistent with the conversion results shown in **Table 2**, and again suggest a problem with the RGDCox-2L vectors developed to date.

Table 3. Cytotoxicity of Ad infected DU145 human prostate cancer cells (10 MOI) exposed to 5-FC expressed as IC_{50} (nM).

Vector	DU145
Uninfected	165.9
AdCMVCD	31.0
AdCMVCDCMVSSTr2	3.4
AdRGDCMVCD	2.2
AdCox-2LCDCox-2LSSTr2	30.4
AdRGDCox-2LCD	114.9
AdRGDCMVCDCMVSSTr2	0.9
AdRGDCox-2LCDCox-2LSSTr2	128.1

In the coming year, we will continue our efforts to produce RGD modified Cox-2L CD and SSTr2 two-gene vectors in a new vector backbone to achieve the highest levels of tumor SSTr2 and CD expression in local and metastatic *in vivo* prostate cancer tumor models after intratumoral or intravenous delivery of the vector. The transduction of the tumor nodules will be evaluated by radiolabeled peptide uptake in the tumor and other normal tissues. We will also evaluate therapeutic efficacy using the newly derived vectors in combination with 5-FC and radiation therapy.

KEY RESEARCH ACCOMPLISHMENTS

- Validated the expression of SSTr2 and CD in tumor cells and xenografts after infection with Ad vectors expressing SSTr2 and CD under control of Cox-2L promoter.
- Determined the conversion of 5-FC to 5-FU in prostate cancer cells and tumor xenografts.
- Demonstrated cytotoxicity of prostate cancer cells infected with Ad vectors under control of Cox-2L promoter expressing CD and exposed to 5-FC.
- Demonstrated localization of ^{99m}Tc-P2045 which binds to SSTr2 in tumor xenografts infected with SSTr2 expressing Ad vectors

REPORTABLE OUTCOMES

Developed new Ad vectors expressing both SSTr2 and CD under control of CMV and Cox-2L promoters.

CONCLUSIONS

In conclusion, the combination of the therapeutic genes CD and SSTr2 with the Cox-2L promoter should provide specificity for tumor uptake of radiolabeled peptides that bind to SSTr2 and selective 5-FC molecular chemotherapy. Once the RGD modified vectors are developed and tested *in vitro*, they will be evaluated in local and metastatic prostate cancer models in the next project period.

ABBREVIATIONS

Ad	adenoviral
CD	cytosine deaminase
Cox-2	cyclooxygenase-2
CMV	cytomegalovirus
5-FC	5-fluorocytosine
5-FU	5-fluorouracil
SSTr2	somatostatin receptor subtype 2

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1. Zinn KR, Chaudhuri TR, Buchsbaum DJ, Mountz JM, and Rogers BE. Detection and measurement of *in vitro* gene transfer by gamma camera imaging. *Gene Ther*, 8:291-299, 2001.
2. Zinn KR, Chaudhuri TR, Buchsbaum DJ, Mountz JM, and Rogers BE. Simultaneous evaluation of dual gene transfer to adherent cells by gamma-ray imaging. *Nucl Med Biol*, 28:135-144, 2001.
3. Della Manna DL, Yamamoto M, Krasnykh V, Zinn KR, Davydova J, Chiz S, and Buchsbaum DJ. New adenoviral vectors for molecular chemotherapy of prostate cancer. *Mol Ther*, 7:S315-S316, 2003.

Appendix Cover Sheet

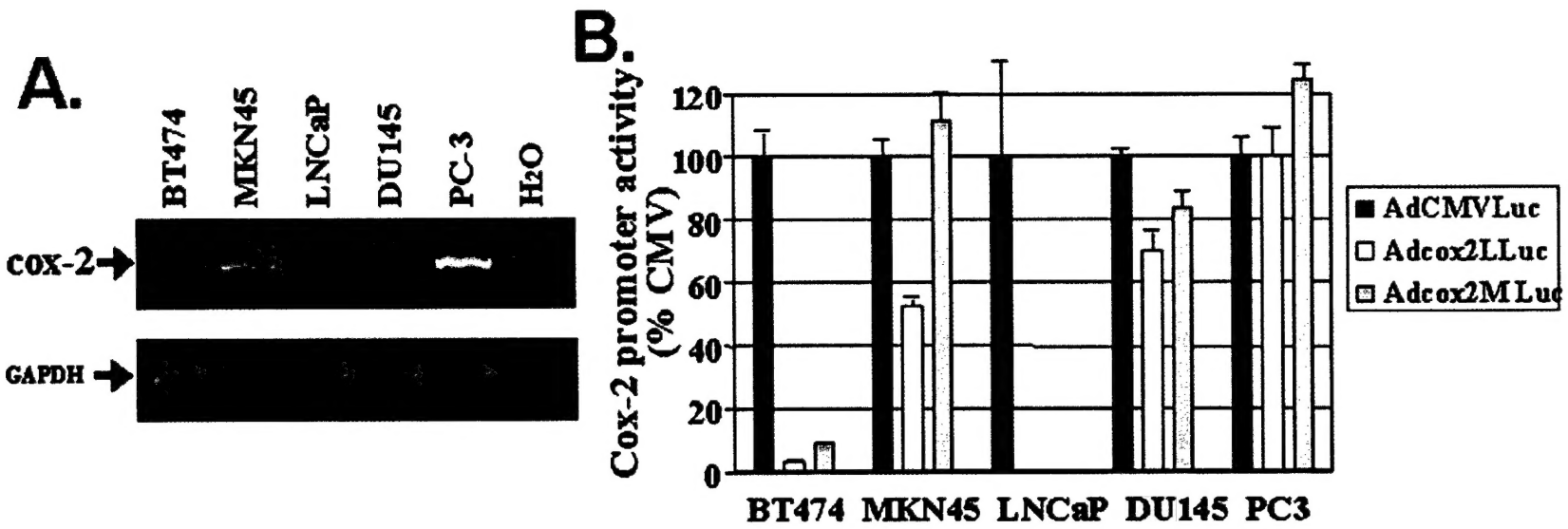


Figure 1. Cellular Cox-2 mRNA status and promoter activity in prostate cancer cells.

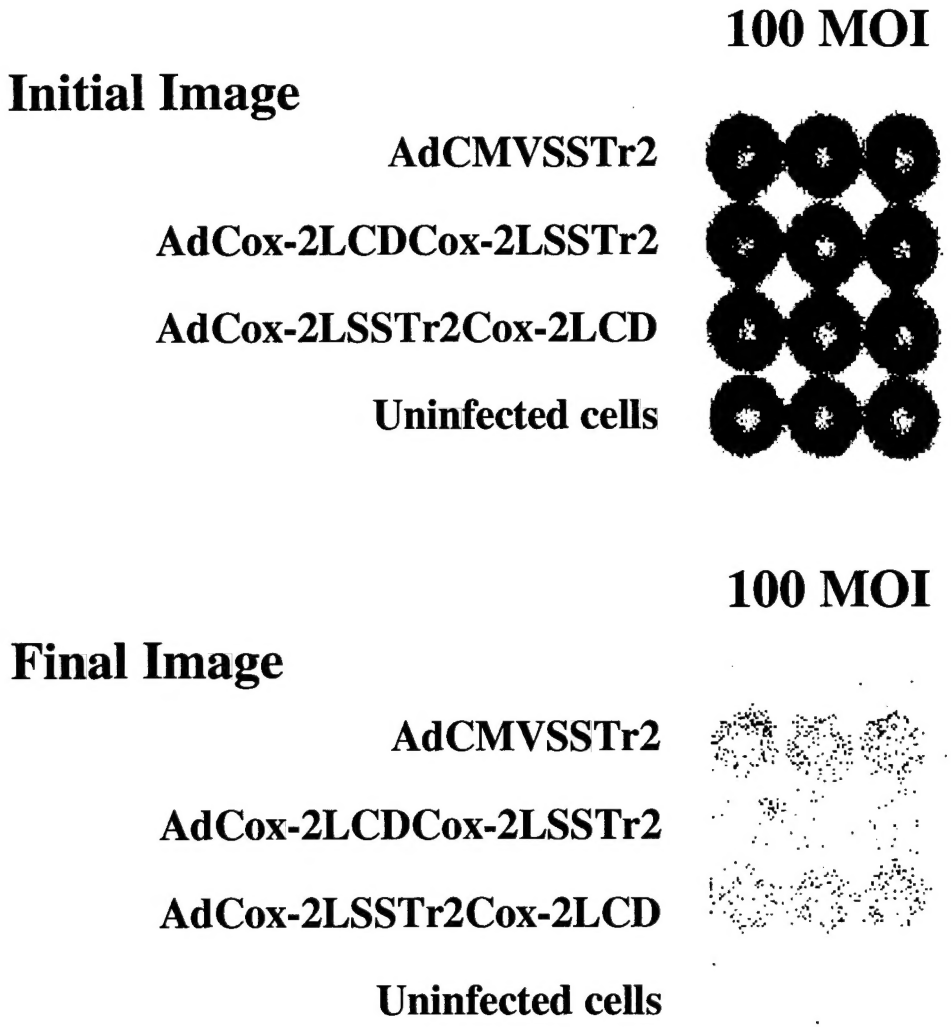


Figure 2. Binding of ^{99m}Tc-P2045 to DU145 prostate cancer cells before (initial) and after (final) washing of cells detected by gamma camera imaging.

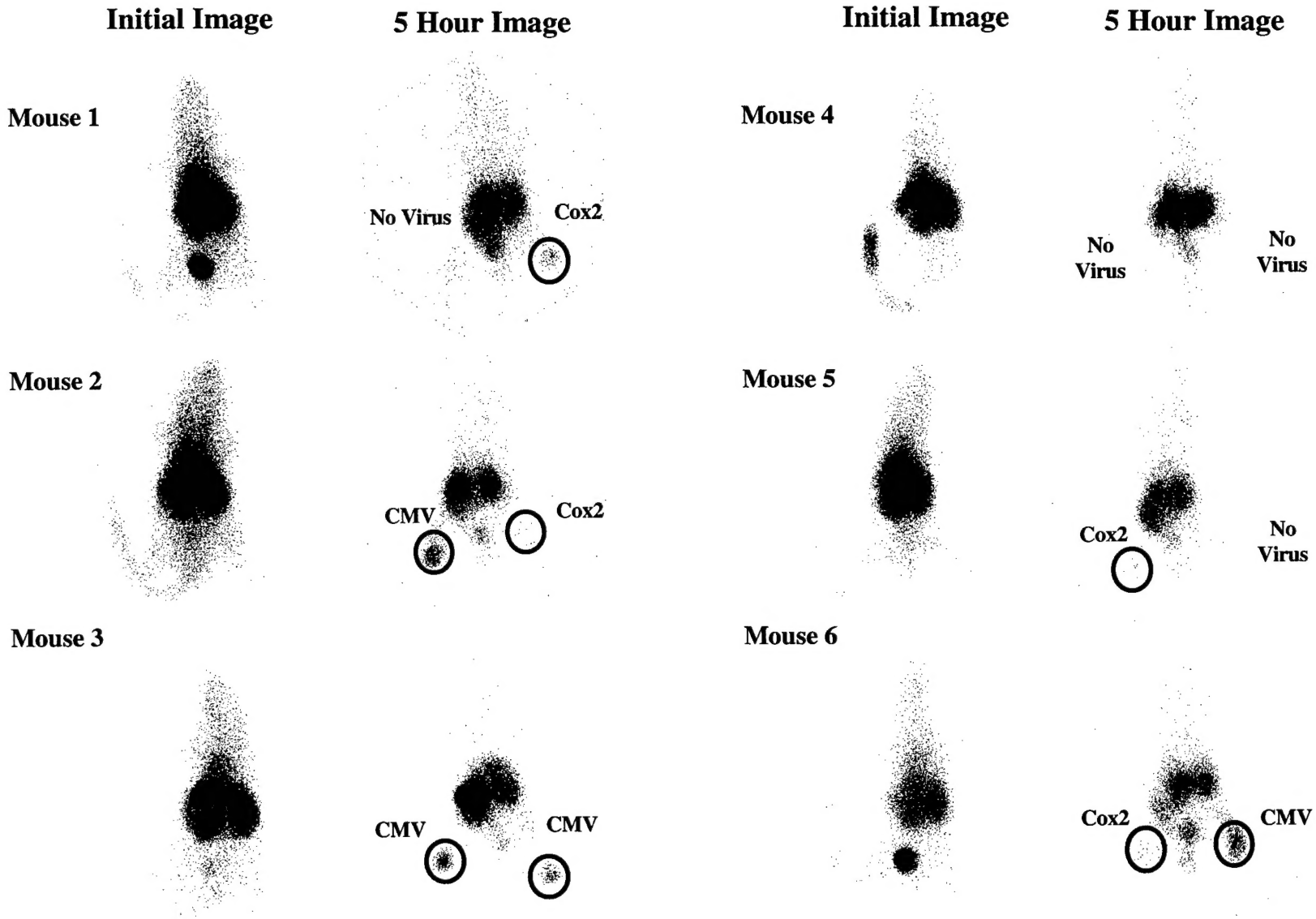


Figure 3. Gamma camera images of mice bearing DU145 prostate cancer xenografts injected intratumorally with AdCMVSSTr2 or AdCox-2LSSTr2 followed 2 days later by *i.v.* administration of ^{99m}Tc-P2045.

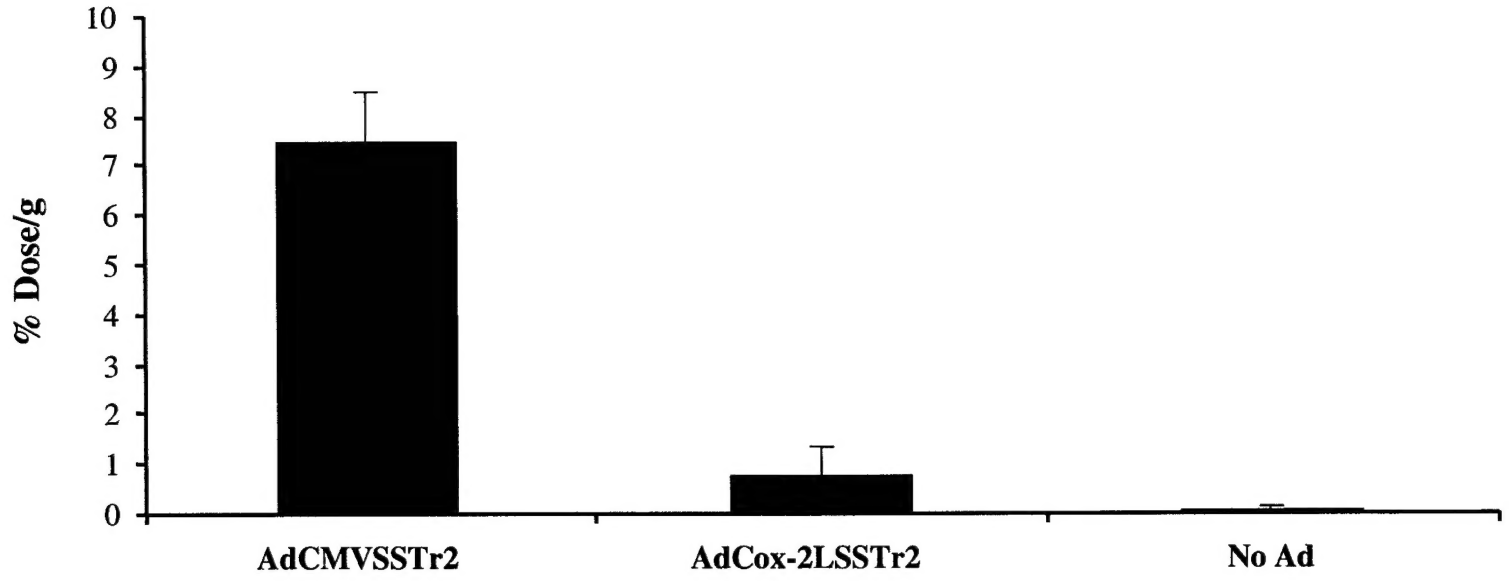


Figure 4. ^{99m}Tc-P2045 localization in DU145 prostate cancer xenografts injected intratumorally with AdCMVSSTr2 or AdCox-2LSSTr2.

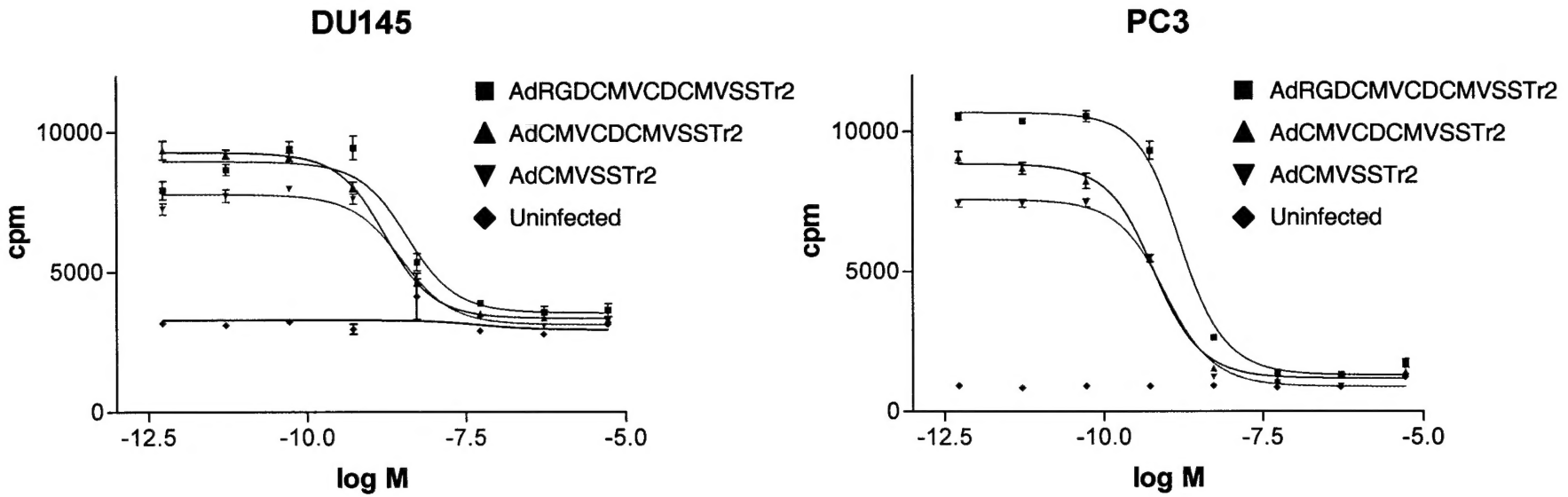
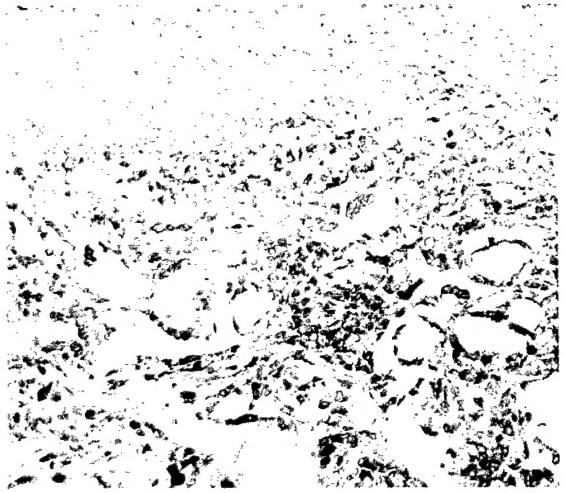
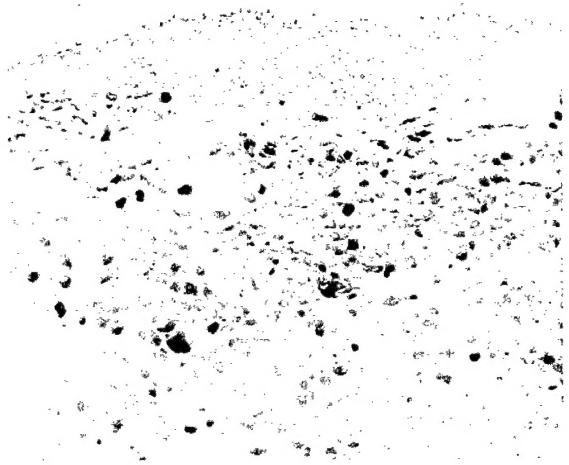


Figure 5. Scatchard analysis of DU145 and PC3 human prostate cancer cells 48 h after infection determined with ^{125}I-somatostatin.

AdCMVCD



AdCox-2LCDCox-2LSSTr2



AdCox-2LSSTr2Cox-2LCD

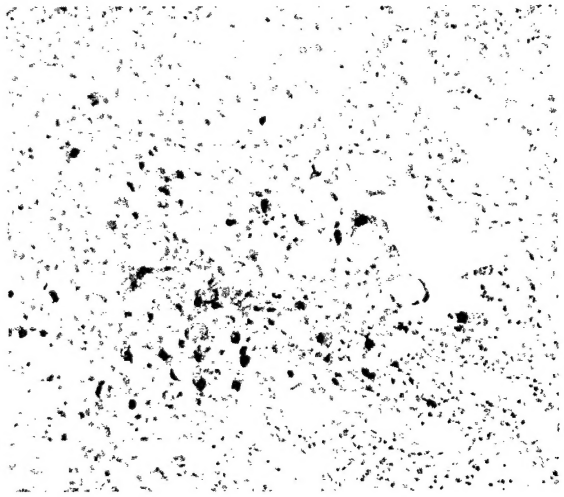


Figure 6. Expression of CD in DU145 prostate cancer xenografts after infection with several Ad vectors.

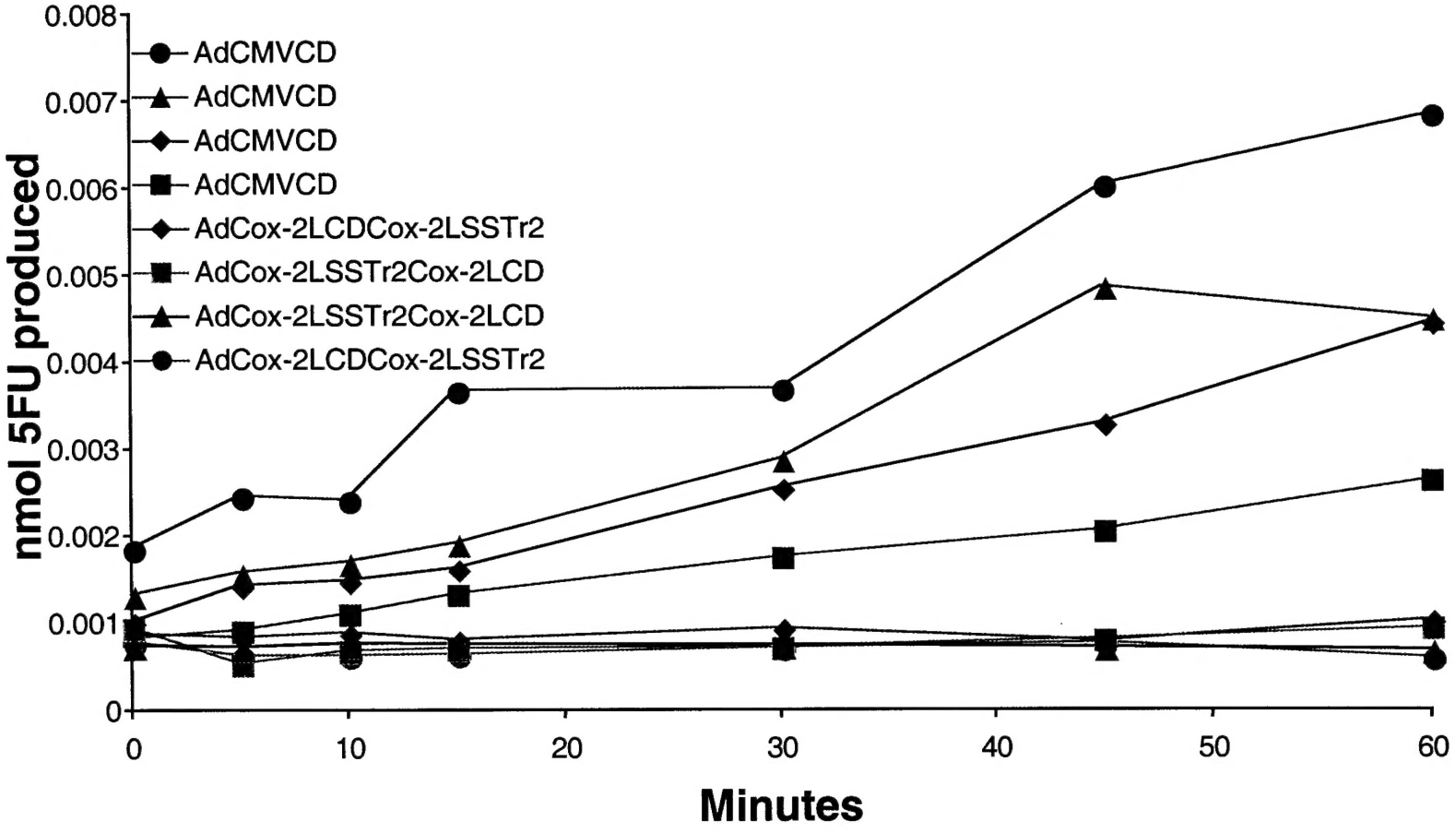


Figure 7. Conversion of 5-FC to 5-FU in homogenized DU145 tumor xenografts 48 h after intratumoral injection of $1x10^9$ pfu AdCMVCD, AdCox-2LCDCox-2LSSTr2, or AdCox-2LSSTr2Cox-2LCD.